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# Complete mitochondrial genome of the frillneck lizard (Chlamydosaurus kingii, Reptilia; Agamidae), another squamate with two control regions

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## Abstract

AbstractUsing PCR, the complete mitochondrial genome was sequenced in three frillneck lizards (Chlamydosaurus kingii).The mitochondria spanned over 16,761 bp. As in other vertebrates, two rRNA genes, 22 tRNA genes and 13 protein codinggenes were identified. However, similar to some other squamate reptiles, two control regions (CRI and CRII) were identified,spanning 801 and 812 bp, respectively. Our results were compared with another Australian member of the family Agamidae,the bearded dragon (Pogana vitticeps). The overall base composition of the light-strand sequence largely mirrored thatobserved in P. vitticeps. Furthermore, similar to P. vitticeps, we observed an insertion 801 bp long between the ND5 and ND6genes. However, in contrast to P. vitticeps we did not observe a conserved sequence block III region. Based on a comparisonamong the three frillneck lizards, we also present data on the proportion of variable sites within the major mitochondrialregions.



## Keywords

Complete, mitochondrial, genome, frillneck, lizard, Chlamydosaurus, kingii, Reptilia, Agamidae, another, squamate, two, control, regions


## Disciplines

Life Sciences | Physical Sciences and Mathematics | Social and Behavioral Sciences


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FULL-LENGTH RESEARCH ARTICLE

# Complete mitochondrial genome of the frillneck lizard (*Chlamydosaurus kingii*, Reptilia; Agamidae), another squamate with two control regions

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**Abstract**

Using PCR, the complete mitochondrial genome was sequenced in three frillneck lizards (*Chlamydosaurus kingii*). The mitochondria spanned over 16,761 bp. As in other vertebrates, two rRNA genes, 22 tRNA genes and 13 protein coding genes were identified. However, similar to some other squamate reptiles, two control regions (CRI and CRII) were identified, spanning 801 and 812 bp, respectively. Our results were compared with another Australian member of the family Agamidae, the bearded dragon (*Pogana vitticeps*). The overall base composition of the light-strand sequence largely mirrored that observed in *P. vitticeps*. Furthermore, similar to *P. vitticeps*, we observed an insertion 801 bp long between the *ND5* and *ND6* genes. However, in contrast to *P. vitticeps* we did not observe a conserved sequence block III region. Based on a comparison among the three frillneck lizards, we also present data on the proportion of variable sites within the major mitochondrial regions.

**Keywords:** *Complete mtDNA, control region, lizard, reptile*

## Introduction

Possessing its own extra-chromosomal genome, the mitochondria have been the workhorse of the molecular revolution in ecology and evolution over the past four decades (Randi 2000). Mitochondrial DNAs (mtDNAs) of vertebrates are 16–18 kbp double-stranded circular molecules, which encode 24 structural genes (two rRNAs and 22 tRNAs) and 13 respiratory protein coding genes together with the control region (CR; Boore 1999). The latter non-coding region accommodates the machinery of mtDNA replication (Reyes et al. 1998). Recent studies have clearly demonstrated the value of using the complete mitochondrial nucleotide sequence when analysing phylogenetic relationships and ancient radiations (Reyes et al. 1998; Janke et al. 2001; Rest et al. 2003; Kumazawa 2004, 2007; Pereira and Baker 2006). However, compared with other vertebrate taxa such as fish, where the complete mitochondrial sequences of approximately 300 species have been reported (Oh et al. 2007), less than 20 complete mitochondrial genomes have been described in squamate reptiles (Kumazawa 2007). In the present study we therefore provide data of the complete mitochondrial genome of an additional squamate reptile, the frillneck lizard *Chlamydosaurus kingii*, and compare its mtDNA genome with another Australian member of the family Agamidae, the bearded dragon (*Pogana vitticeps*).

## Materials and methods

### *Study species*

The frillneck lizard (*C. kingii*) inhabits tropical savanna woodlands throughout the northern part of Australia and southern New Guinea. It is a large

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arboreal lizard, with a total length of up to 90 cm (Cogger 2002). The results presented in this study are based on sequencing the complete mtDNA of three lizards collected in our study area situated approximately 60 km south-east of Darwin in the Northern Territory of Australia.

*DNA extraction, amplification and sequencing*

Genomic DNA was isolated from whole blood by phenol–chloroform extraction. Published sequences of *P. vitticeps* and *C. kingii* [GenBank accession numbers NC_006922 and AF128469, respectively, (Macey et al. 2000; Amer and Kumazawa 2005)] were used to design specific primers (Table I). The PCR reactions were performed in a total volume of 20 μl containing 100 ng total genomic DNA, 1 U recombinant Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA), 0.125 mM each nucleotide, 2 μl of 10 × PCR buffer (Invitrogen) and 0.6 μM each primer. The $MgCl_2$ concentrations were adjusted according to the different primer combinations. The PCR amplifications were conducted as follows: 94°C/3 min + (94°C/30 s or +50 − 62°C/30 s, depending on primer combination, +72°C/1 min) × 30 + 72°C/10 min. The PCR products were purified with ExoSap (GE Healthcare, Piscataway, NJ, USA), and were sequenced from both directions on an ABI 3130*xl* Genetic Analyzer using BigDye Terminator Kit V.3.1 (Applied Biosystems, Foster City, CA, USA). DNA sequences were aligned using BioEdit (Hall 1999) and ClustalW (Chenna et al. 2003). All sequences are deposited in NCBI Genbank (accession numbers EF090421–EF090423).

## Results and discussion

The complete nucleotide sequence of the three frillneck lizards spanned over 16,761 bp, 10 bp longer than that recorded in *P. vitticeps* (Amer and Kumazawa 2005). As in other vertebrates, two rRNA genes, 22 tRNA genes and 13 protein coding genes were identified (Figure 1). Furthermore, the relative position and orientation of all the genes were identical to most other vertebrates, with one exception; similar to *P. vitticeps* (Amer and Kumazawa 2005) and other acrodont lizards (Macey et al. 2000), the IQM tRNA gene cluster was arranged into QIM (Figure 1). The overall base composition of the light-strand sequence of the three frillneck lizards largely mirrored that observed in *P. vitticeps* by Amer and Kumazawa (2005): A, 34.4 vs. 33%; T, 24.2 vs. 24%; C, 29 vs. 29.9%; and G, 12.4 vs. 13.2%, respectively.

Similar to *P. vitticeps*, we observed an insertion 801 bp long between the *ND5* and *ND6* genes (Figure 1). Apart from being 11 bp shorter (2 bp missing at the 5′ end and 9 bp missing the 3′ end), the sequence had an identical nucleotide sequence to the major noncoding region between $tRNA^{Pro}$ and $tRNA^{Phe}$. To ensure that the two similar regions were not artefacts and/or a result of misinterpretation of the sequencing data, we used numerous different primer combinations to amplify the junctions between the two CRs and their adjacent genes (for CRI: *ND5*, 13191F; *ND5*, 13490F; *ND5*, 12920F and CRI, 14410R; *Cytb*, 14935R; *Cytb*, 15230R; and for CRII: *Cytb*, 15485F; *Cytb*, 15230F; *tRNAPro*, 15910F; CRII, 16200F and 12S, 155R; 12S, 279R; 12S, 556R). In the frill neck lizards, both of these sequences included conserved sequence blocks I and II, and hence we identified them as CRs. However, in contrast to *P. vitticeps* we did not observe a conserved sequence block III region. Furthermore, in *P vitticeps* the two regions had an identical length, each spanning 798 bp (Amer and Kumazawa 2005). Thus, as recorded in some other reptile taxa such as *P. vitticeps*, the mitochondrial genome of the frillneck lizard contained two CRs (Figure 1), situated at the same positions as in the former taxa (Amer and Kumazawa 2005). Our results also confirm the data presented by Amer and Kumazawa (2005) (based on a PCR-mediated assay and partial sequencing) of an insertion of a duplicate CR between the *ND5* and *ND6* genes in Australian agamid lizards.

In snakes, the possession of two functional CRs has been suggested to be advantageous by increasing genome replication rates (Jiang et al. 2007). As snakes, like other reptiles, are ectotherms, Jiang et al. (2007) suggested that "transcriptional decoupling via independent CRs could provide a more direct means of counteracting thermodynamic depression of enzymatic rates at low temperatures". However, most reptiles, like frillneck lizards, live in the tropics, where a recent study has shown that these animals are able to maintain high and stable body temperatures throughout the year (Shine and Madsen 1996). Furthermore, duplicated controls regions have also been documented in birds (Mindell et al. 1998). It is therefore unlikely that the duplication of the CR in reptiles is an adaptation to counteract the effects of thermodynamic depression. Thus, we agree with a statement made by Kumazawa and Endo (2004) that "the mechanisms for the maintenance of apparently redundant CRs, as well as those for the concerted sequence evolution of the two CRs within species, remain to be elucidated".

The CR in birds (for example, Kvist et al. 1998) and mammals (for example, Bickham et al. 1996) often exhibit high nucleotide polymorphism. However, in some squamate reptiles this part of the mitochondrial genome is highly conserved (Burbrink et al. 2000; Ujvari et al. 2005). Indeed, although our analyses are only based on three individual lizards, the proportion of variable sites among the individuals revealed that also in frillneck lizards the two CRs showed lower levels of polymorphism than, for

Table I. Primers designed for sequencing frillneck lizard mtDNA.

| Primer identifier | Sequence (5′–3′) | Chain |
|---|---|---|
| 12S-115F | CAAGCATCCACACCCCAGT | H |
| 12S-115R | ACTGGGGTGTGGATGCTTG | L |
| 12S-279R | TTGAGTCCTTGAAGCCGTTTA | L |
| 12S-556F | ACTCAAAAGACCTGGCGGTA | H |
| 12S-556R | TACCGCCAGGTCTTTTGAGT | L |
| 12S-855F | CTGATCTGAAACCACGCTCT | H |
| 12S-855R | AGAGCGTGGTTTCAGATCAG | L |
| 16S-1740F | TTACTTAATACATGCAACCAACT | H |
| 16S-1740R | AGTTGGTTGCATGTATTAAGTAA | L |
| 16S-1995F | TCTCTTTAGGTAGGTCAATGAA | H |
| 16S-1995R | TTCATTGACCTACCTAAAGAGA | L |
| 16S-2310F | ATGGTGCAGAAGCTATTAAGG | H |
| 16S-2310R | CCTTAATAGCTTCTGCACCAT | L |
| ND1-2690F | ACTCATAGCGATTGCCTTCTT | H |
| ND1-2690R | AAGAAGGCAATCGCTATGAGT | L |
| ND1-3040F | TGCCGTAGCTCAAACCTTAT | H |
| ND1-3040R | ATAAGGTTTGAGCTACGGCA | L |
| ND1-3510F | CATTCCTCCCAATCACCTTAA | H |
| ND1-3510R | TTAAGGTGATTGGGAGGAATG | L |
| ND2-3803F | TTGTCACCAATAGCCCTCTTT | H |
| ND2-3803R | AAAGAGGGCTATTGGTGACAA | L |
| ND2-3945F | AACAGAAGCCGCAACAAAAT | H |
| ND2-4447F | CTACTACTAACATCCTCATCAT | H |
| ND2-4447R | ATGATGAGGATGTTAGTAGTAG | L |
| COI-5185F | AATCGATGATTCCTATCCACAA | H |
| COI-5185R | TTGTGGATAGGAATCATCGATT | L |
| COI-5390F | TAGTAATACCAATCATGATTGGA | H |
| COI-5390R | TCCAATCATGATTGGTATTACTA | L |
| CoxI-5979F | CCTTCGGCTATATAAGCATAGT | H |
| CoxI-5979R | ACTATGCTTATATAGCCGAAGG | L |
| COI-6255R | TCGTGTCAATAGAAGAGTTTGA | L |
| CO1-6560F | CTAGTCAGCGCTCTTACCAT | H |
| CO1-6560R | ATGGTAAGAGCGCTGACTAG | L |
| CO1-6563F | TTTACCAGCGCTATCTCCATA | H |
| CO1-6563R | TATGGAGATAGCGCTGGTAAA | L |
| CO2-7523F | AGCTTCACTATTCAACAACTCT | H |
| CO2-7523R | AGAGTTGTTGAATAGTGAAGCT | L |
| ATP6-7660F | CCTGAATAACAATAATCCTTCTT | H |
| ATP6-7660R | AAGAAGGATTATTGTTATTCAGG | L |
| AT6-7780F | CCCAATTTTCAATCCCAACTG | H |
| AT6-7780R | CAGTTGGGATTGAAAATTGGG | L |
| ATP6-8140F | CCAACCAAAACAATAGCCCAT | H |
| ATP6-8365F | CATCCTAGAAATCGCAGTAGCTA | H |
| ATP6-8365R | TTTAGCTACTGCGATTTCTAGGA | L |
| CO3-9150F | AAGCAGCTGCCTGATACTGA | H |
| CO3-9150R | TCAGTATCAGGCAGCTGCTT | L |
| CO3-9260F | TGTGGCTTCCAACCACACAA | H |
| CO3-9260R | TTGTGTGGTTGGAAGCCACA | L |
| CO3-9440F | TACCCTTCTCCCTACAATTCT | H |
| CO3-9440R | AGAATTGTAGGGAGAAGGGTA | L |
| ND4-10055R | TATGCTGTAATTGYGGGTTGTA | L |
| ND4-10300F | CTACTAATAATTGCCCTATCAA | H |
| ND4-10300R | TTGATAGGGCAATTATTAGTAG | L |
| ND4-11090F | CCCAACAATTAATTTAATAGGAG | H |
| ND4-11090R | CTCCTATTAAATTAATTGTTGGG | L |
| ND4-11200F | AGCATACACCATACACATATCT | H |
| tRNAHis-11390F | TAGGTCGTGACCCTAAAGATA | H |
| tRNAHis-11390R | TATCTTTAGGGTCACGACCTA | L |
| tRNAHis-11730F | AAGTCCAACCTGTTTCAGTAT | H |
| tRNAHis-11730R | ATACTGAAACAGGTTGGACTT | L |

Table I – *continued*

| Primer identifier | Sequence (5′–3′) | Chain |
|---|---|---|
| ND5-12060F | CAACCGAATTGGAGACATCG | H |
| ND5-12060R | CGATGTCTCCAATTCGGTTG | L |
| ND5-12460F | AGCCAACTAGGACTAATAATA | H |
| ND5-12460R | TATTATTAGTCCTAGTTGGCT | L |
| ND5-12500F | GCTCACCCGGACTAGCTAT | H |
| ND5-12500R | ATAGCTAGTCCGGGTGAGC | L |
| ND5-12920F | TTAGGGCTATTACTCTCAACAA | H |
| ND5-12920R | TTGTTGAGAGTAATAGCCCTAA | L |
| ND5-13191F | TGACTAGAAAAATCAGGACCA | H |
| ND5-13191R | TGGTCCTGATTTTTCTAGTCA | L |
| ND5-13490F | TGTAAACCTATGCCCTATGTAT | H |
| ND5-13490R | ATACATAGGGCATAGGTTTACA | L |
| CR1-13850F | CCAAGACCTATGGTTGATGG | H |
| CR1-13850R | CCATCAACCATAGGTCTTGG | L |
| CR1-14030R | TGTCATACGAGCATTGAAAGAT | L |
| CR1-14410F | TCTGAAGCAAGAGCAATCGAA | H |
| CR1-14410R | TTCGATTGCTCTTGCTTCAGA | L |
| Cytb-14920F | AAAACMTYCACGCAAACGGRG | H |
| Cytb-14935R | CYCCGTTTGCGTGRAKGTTTT | L |
| Cytb-15230F | TACACTTCATCATACCATTTGTA | H |
| Cytb-15230R | TACAAATGGTATGATGAAGTGTA | L |
| Cytb-15480R | GGTTGGGTTGTTGGAKCCTG | L |
| Cytb-15485F | ACATCAAACCGGAGTGATACT | H |
| Cytb-15485R | AGTATCACTCCGGTTTGATGT | L |
| tRNAPro-15910F | TGGCCCCCAAAGCCAACAT | H |
| tRNAPro-15910R | ATGTTGGCTTTGGGGGCCA | L |
| CR2-16200F | TTGCTCTAACTACATGTCATTAT | H |
| CR2-16200R | ATAATGACATGTAGTTAGAGCAA | L |

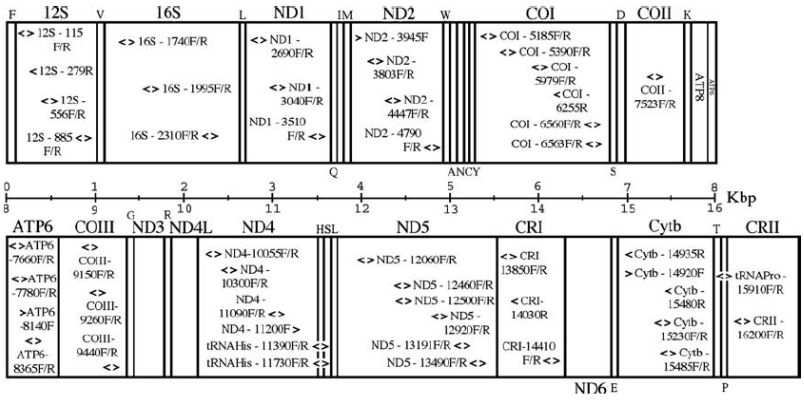


Figure 1. Linearised representation of the complete mitochondria region in the frillneck lizard. *Note*: Location and orientation of primers used are depicted by arrows. See Table I for specific primer sequences. Transfer RNA genes are abbreviated as the corresponding one-letter amino acid codes.

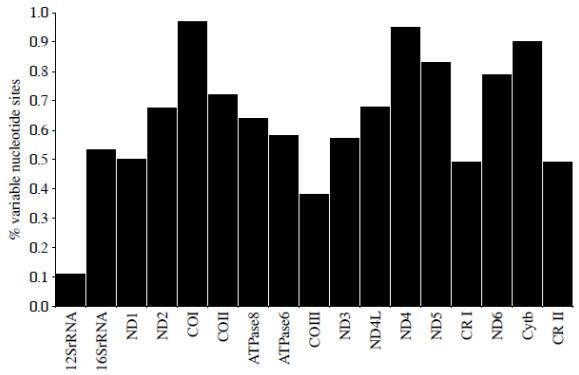


Figure 2. Proportion of variable sites (0–1%) among three frillneck lizard's complete mitochondria.

example, the *COI*, *ND4* and *Cytb* genes (Figure 2). The difference in polymorphism suggests that the CRs in frillneck lizards exhibit slower evolutionary rates compared with the three latter genes. Thus, as proposed by Ujvari et al. (2005), population genetic diversity-based CR polymorphism (and management decisions from such estimates) may depend as much on the taxa being investigated as upon the underlying pattern of genetic variation within the study population.

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